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Polytene chromosomes of *Simulium* (*Wilhelmia*) *xingyiense* (Diptera: Simuliidae) from China

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Abstract: A standard photographic map of *Simulium* (*Wilhelmia*) *xingyiense* Chen and Zhang was constructed from larval salivary gland polytene chromosomes and is described herein. Polytene chromosomes from wild larvae collected from three populations in Guiyang, Guizhou Province, China were analyzed. By using an upgraded method of preparation, the polytene chromosomes of the larval salivary glands of *S.* (*W.*) *xingyiense* were observed and analysed. The results indicated that this species has three pairs of chromosomes (2n = 6) which are arranged from the longest to the shortest. Chromosome Ⅰ is metacentric while the chromosome Ⅱ and III are submetacentric. The nucleolar organizer is situated near the centromeric band of the short arm of chromosome Ⅰ. The Balbiani ring and double bubble are located about the middle of short arm of chromosome Ⅱ. The pericentric heterochromatin of all chromosome arms forms an evident chromocenter. High inversion polymorphism is observed, with 0.64 inversions per individual. Thus, the polytene chromosomes of *S.* (*W.*) *xingyiense* are species-specific. Inversion is one of the characteristics of polytene chromosomes of *S.* (*W.*) *xingyiense*.

Key words: *Simulium* (*Wilhelmia*) *xingyiense*; polytene chromosome; chromosome map; karyotype; inversion polymorphism

1 INTRODUCTION

Blackflies are blood-sucking insects representing a compact distinct cosmopolitan family of Diptera. Many blackfly species are vectors of pathogens of dangerous diseases such as Onchocerciasis, Leucocytozoonsis, Mansonelliasis, Trypanosomiasis and Dirofilariasis (Adler, 2005). Blackflies has been studied at the morphotaxonomic level in various parts of the world (Crosskey, 1960; Datta 1974a, 1974b; Davies, 1968; Takaoka, 1977, 1979; Takaoka and Davies, 1995; Takaoka and Adler, 1997; Chen, 2003). Most blackflies are recognized and defined by external morphological characters of larvae, pupae and adults. However, some blackflies are difficult to classify because their morphology is similar among species (Crosskey, 1981), especially in the sibling species (Procunier and Muro, 1993). More than 200 species have been identified from China on the basis of morphological characters (Chen, 2003).

Larval polytene chromosomes of blackflies (Simuliidae) have been proved to be an excellent material not only for the studies of cytotaxonomy (Vajime and Dunbar, 1975; Rothfels, 1979; Dunbar and Vajime, 1981; Procunier and Muro, 1993), chromosomal polymorphisms (Bedo, 1979; Rothfels and Freeman, 1977), phylogenetic relationships (Bedo, 1979; Rothfels, 1981), but also for identifying members of sibling species complexes (Bedo, 1977; Conn *et al.*, 1989; Boakye, 1993; Hadi *et al.*, 1996c).

Knowledge of cytotaxonomy of blackflies from China is very scarce, and only three species have been studied cytogenetically (Wen *et al.*, 2007a, 2007b; Zhang *et al.*, 2008). No published chromosomal maps are available for members of the subgenus *Wilhelmia* in China, although maps are available for about 9 nominal species of the subgenus in the USSR (Chubareva and Petrova 2003, 2007). *Simulium* (*Wilhelmia*) *xingyiense*, originally described from Xingyi by Chen and Zhang (1998), is a peculiar species in China, which belongs to the subgenus *Wilhelmia* of the genera *Simulium*. Here we present the standard photographic maps of the larval polytene chromosomes for *S.* (*W.*) *xingyiense* collected from Guiyang, Guizhou Province, China.

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2 MATERIAL AND METHODS

2.1 Test insects

Larval specimens of *S.* (*W.*) *xingyiense* were collected from three locations at different altitudes of Guiyang, Guizhou Province (Table 1). Larvae were picked up by small forceps off submerged fallen leaves and trailing water glasses. They were preserved in freshly prepared Carnoy's fixative (absolute ethanol: glacial acetic acid = 2:1, v/v). The fixative was changed twice during the first hour and the preserved larvae were then stored in a freezer (−20℃) until required for slide preparation.

Larval identification was based on the shape of the postgenal cleft, respiratory histoblasts and head pattern (Chen and Zhang, 1998).

Table 1 Collection data for *Simulium* (*Wilhelmia*) *xingyiense* in Guiyang, Guizhou, China

Collecting location	Collecting date (year-month)	Latitude (N)	Longitude (E)	Altitude (m)
Huaxi Park	2007-05	26.4321	106.6638	1 100
Qingyan Stream	2009-06	26.3348	106.6896	1 040
Yudong Stream	2010-08	26.6368	106.8234	999

2.2 Chromosome preparation and observation

Only last stage or penultimate-instar larvae were used for salivary gland polytene chromosome preparations. The polytene chromosomes were stained with carbol fuchsin for 15 − 20 min using the methods described by Huang *et al.* (2009). Well spread polytene chromosomes from 2 − 3 salivary gland cells per slide were photographed with Leica Technical Pan Film with a green filter (1 000 ×).

2.3 Data analysis

The photographic images were transferred to Adobe Photoshop 7.0 for map assemblage. The relative lengths of the three chromosome pairs and their respective arms were measured from photographs of 10 well spread polytene chromosome complements. Chromosome mapping conventions and nomenclature follow the method described by Bedo (1977).

3 RESULTS

3.1 General characterization of the karyotype of *S.* (*W.*) *xingyiense*

S. (*W.*) *xingyiense* has 3 pairs of chromosomes (2n = 6) which are arranged from the longest to the shortest (Fig. 1). Chromosome Ⅰ is metacentric and chromosome Ⅱ and Ⅲ are submetacentric. Homologs are tightly conjugated. The nucleolus is associated with the short arm of chromosome Ⅰ. Pericentric heterochromatin consists of thin, intensely stained bands.

The major peculiarity of the *S.* (*W.*) *xingyiense* karyotype is the fusion of pericentric heterochromatin of all chromosome arms. As a result an evident intensely stained chromocenter is formed in 185 nuclei out of 204 examined nuclei from 102 larvae, from which 6 arms diverge radially. The chromocenter presents two kinds of appearances. In a few larvae pericentric heterochromatin is completely fused to form a stable tight chromocenter of the *Drosophila* type and represented by large heterochromatin blocks (Fig. 2: A). However, in most larvae the bands of all six arms of polytene chromosomes arrange to form a loose ring-like "pseudochromocenter" (Fig. 2: B). This structure is typical of this study, accounting for about 96%.

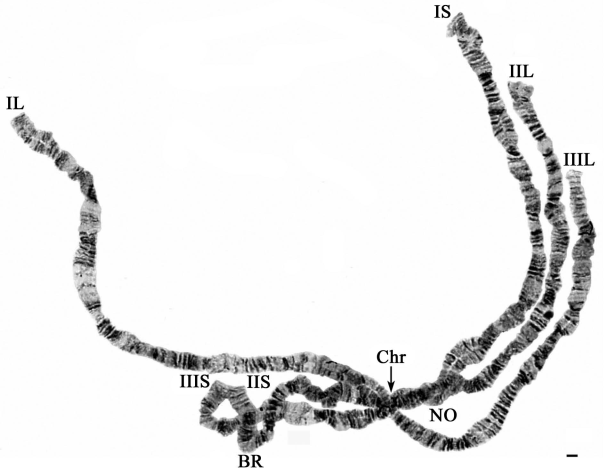

Fig. 1 Photographic map of salivary gland polytene chromosome complement of *Simulium* (*Wilhelmia*) *xingyiense*

Ⅰ, Ⅱ, Ⅲ: Chromosome Ⅰ, Ⅱ and Ⅲ, respectively; S: Short arm; L: Long arm; Chr: Chromocenter; NO: Nucleolus; BR: Balbiani ring. Scale bar = 10 μm.

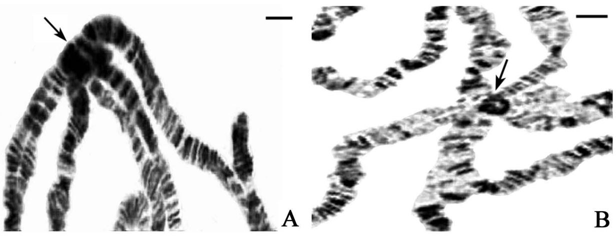

Fig. 2 The block (A) and ring (B) of chromocenter in *Simulium* (*Wilhelmia*) *xingyiense*

Scale bar = 25 μm.

A high frequency of heterozygous inversions is revealed for *S.* (*W.*) *xingyiense*. Sixty-five heterozygous inversions have been found in 204 nuclei out of 102 larvae examined (Fig. 3). On average, inversion number is 0.64 per larva. These inversions are mainly distributed in IS, IL, IIL and IIIL (Table 2).

3.2 Morphological features of polytene chromosomes in *S.* (*W.*) *xingyiense*

The mean relative lengths of the polytene chromosome arms and the number of sections assigned to each arm in the standard map are shown in Table 3. As already noted, the arms are designated according to their specific markers characteristic of the standard karyotype of the genus *Simulium* (Rothfels *et al.*, 1978). The details of polytene chromosomes of *S.* (*W.*) *xingyiense* are described below.

Table 2 Frequency of heterozygous inversions in *Simulium* (*Wilhelmia*) *xingyiense* populations

Heterozygous inversion	Frequency (%)
IS	5.48
IL	6.85
IIL	9.58
IIIL	0.68

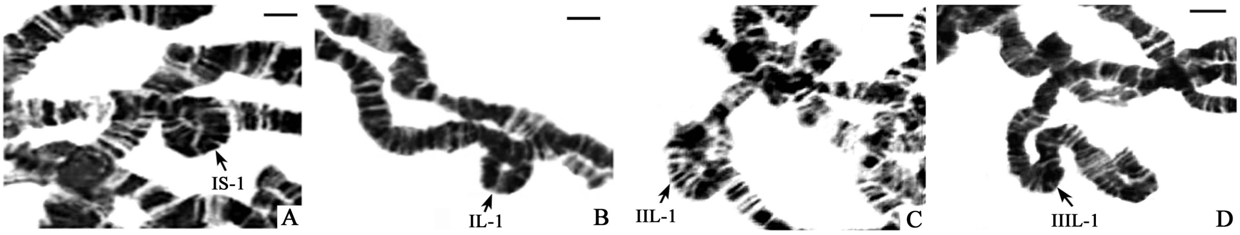

Fig. 3 Heterozygous inversions in *Simulium* (*Wilhelmia*) *xingyiense*
A: Inversion IS-1; B: Inversion IL-1; C: Inversion IIL-1; D: Inversion IIIL-1. Scale bar = 25 μm.

Table 3 Polytene chromosome lengths measured from 10 nuclei of larval salivary gland cells of *Simulium* (*Wilhelmia*) *xingyiense*

Properties	Chromosome Ⅰ		Chromosome Ⅱ		Chromosome Ⅲ	
	IS	IL	IIS	IIL	IIS	IIIL
% TCL	18.18 ± 1.37	22.04 ± 1.16	12.97 ± 1.21	23.21 ± 1.31	9.33 ± 1.31	14.32 ± 1.42
Number of sections/arm	18	22	13	23	9	15
Arm ratio	1.2 M		1.8 SM		1.5 SM	

TCL: Total complement length (mean ± *SE*); M: Metacentric; SM: Submetacentric.

Chromosome Ⅰ is metacentric and the longest of the complement (Fig. 4). It is 40% TCL, divided into 40 sections (1 – 40). The short arm (IS) (Fig. 4: A) extends from sections 1 – 18 while the long arm (IL) (Fig. 4: B) contains sections 19 – 40. The short arm is characterized by the presence of a nucleolar organizer (NO) in section 17. Telomeric section 1 has a typical banding pattern, with three closely spaced thin bands similar in staining intensity and two wider bands stained more intensely. The Sim-end is a marker characteristic of chromosome Ⅰ in all species of the genus. By this marker, IS is easily distinguishable in the karyotype of any species. Small puffs are detected in sections 8, 11, 15 and 16. Arm IL is marked by a pericentric euchromatic region followed by two bands similar in thickness and staining intensity (section 23). The telomeric region is also euchromatic and has two specific bands. Small puffs are observed in sections 24, 26, 30, 33 and 35.

Chromosome Ⅱ is submetacentric and remarkably shorter than chromosome Ⅰ (Fig. 5). It is divided into 35 sections (41 – 75). Arm IIS (Fig. 5: A) is distinguishable by the Balbiani ring (BR) in section 47 followed by the "double bubble" in section 49, and five pericentric thick bands in section 52. Arm IIL (Fig. 5: B) is characterized by two intensely stained close telomeric marker bands (section 75). Section 53 and 54, which are close to the centromere, are somewhat thicker and lack heterochromatin bands. Distinct puffs are observed in sections 60, 63, 65 and 70.

Chromosome Ⅲ is also submetacentric and the shortest of the complement, and contains sections 76 – 100 (Fig. 6). It is characterized by slightly widened telomeric region (section 76) and two distinct puffs (sections 78 and 80) flanked by dense bands. Moreover, the presence of the 3 dark bands in section 85 adjacent to the centromere is also a useful cytological landmark for the short arm (Fig. 6: A). Arm IIIL (Fig. 6: B) doesn't show easily detectable

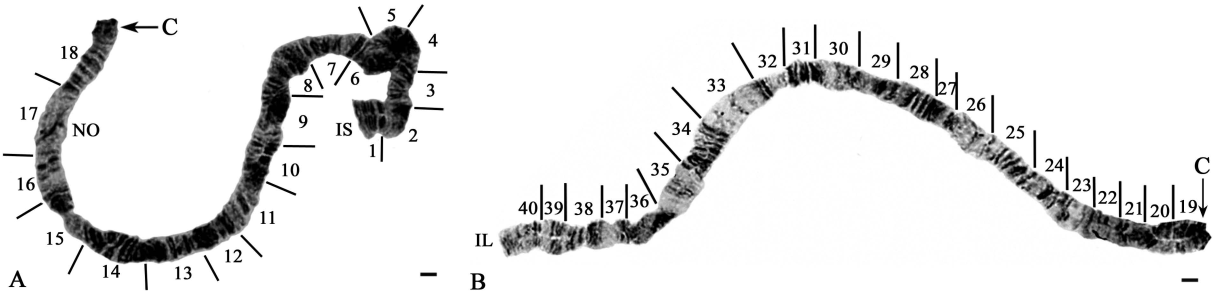

Fig. 4 Photographic map of short arm (A) and long arm (B) of chromosome Ⅰ of *Simulium* (*Wilhelmia*) *xingyiense*
NO: Nucleolar organizer. Scale bar = 10 μm.

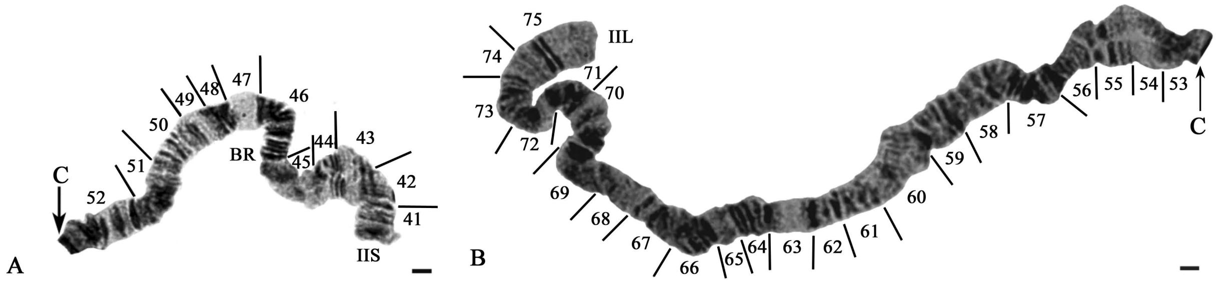

Fig. 5 Photographic map of short arm (A) and long arm (B) of chromosome Ⅱ of *Simulium* (*Wilhelmia*) *xingyiense*
Scale bar = 10 μm.

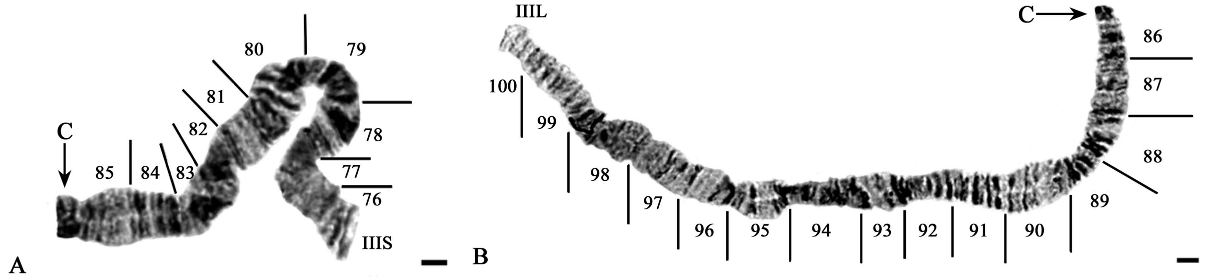

Fig. 6 Photographic map of short arm (A) and long arm (B) of chromosome Ⅲ of *Simulium* (*Wilhelmia*) *xingyiense*
Scale bar = 10 μm.

markers and consists of regularly alternating bands and interbands. Puffs are observed in sections 90, 93, 95 and 96.

4 DISCUSSION

S. (*W.*) *xingyiense* has three pairs of polytene chromosomes (2 n = 6), which is a general pattern of salivary gland polytene chromosomes complement of *Simulium* (Bedo, 1977; Rothfels, 1979; Boakye, 1993; Hadi *et al.*, 1995, 1996a, 1996b, 1996c).

The principal landmarks (*e. g.*, nucleolar organizer, Balbiani ring, and bulge) in *S.* (*W.*) *xingyiense* are found in taxa throughout the Simuliidae (Rothfels, 1979). The nucleolar organizer is in the short arm of chromosome Ⅰ near the centromere, as in the majority of examined species of the subgenus *Wilhelmia*, *e. g.*, *S.* (*W.*) *lineatum*, *S.* (*W.*) *paraequinum*, *S.* (*W.*) *equinum*, *S.* (*W.*) *veltistshevi*, *S.* (*W.*) *salopiense*, and *S.* (*W.*) *pseudequinum*.

The Balbiani ring and the double bubble are located in the short arm of chromosome Ⅱ as reported in *S.* (*W.*) *veltistshevi* (Grinchuk *et al.*, 1975), *S.* (*W.*) *turgaicum* (Grinchuk *et al.*, 1969, 1974), *S.* (*W.*) *lineatum* (Weber and Grunewald, 1989), *S.* (*W.*) *paraequinum* (Petrova *et al.*, 2003), *S.* (*W.*) *equinum* (Weber and Grunewald, 1989), *S.* (*W.*) *pseudequinum* (Chubareva *et al.*, 2007), and *S.* (*W.*) *salopiense* (Chubareva *et al.*, 2007).

In contrast, these features are located in the short arm of chromosomes Ⅲ of species of other subgenera, such as *Nevermannia* Enderlein, *Simulium* Latreille *s. str.* and *Montisimulium* Rubtsov, that is, *S.* (*N.*) *feuerborni* (Kuvangkadilok *et al.*, 1999a), *S.* (*N.*) *caudisclerum*, *S.* (*S.*) *nakhonense* and *S.* (*M.*) sp. G (Kuvangkadilok *et al.*, 1999b). However, there are differences in the arrangement of the Balbiani ring and the double bubble among examined species of the subgenus *Wilhelmia*. In *S.* (*W.*) *xingyiense*, they are located about the middle of chromosome arm IIS as reported in *S.* (*W.*) *lineatum*, *S.* (*W.*) *veltistshevi*, *S.* (*W.*) *salopiense* and *S.* (*W.*) *paraequinum*, whereas in *S.* (*W.*) *equine* and *S.* (*W.*) *pseudequinum* they occur in the distal region of IIS. The results of this study suggest that the positions of the Balbiani ring and the double bubble as well as the banding patterns are specific to the species. Thus, the study of larval polytene chromosome of black flies is an excellent way to identify species in the subgenus *Wilhelmia* that are similar morphologically.

In *S.* (*W.*) *xingyiense*, the main karyotypic character is the distinctive structure of centromere regions connected by thin chromonema fibers into chromocenter. Chromocenter is also found in some species of *Prosimulium*, *Metacnephia*, *Cnephia*, *Nevermannia* and *Wilhelmia* (Chubareva and Petrova, 2003). In the subgenus *Wilhelmia*, six species out of nine karyotypically described species which are mostly from the USSR have chromocenter. Hence, the appearance of chromocenter is impossibly characteristic of the entire subgenus *Wilhelmia*.

According to Rubzov (1956), the subgenus *Wilhelmia* is distinctly divided into three species groups: *equinum*, *salopiense* and *sergenti* by morphological characters, with the last including only two species (from Algeria and Spain). *S.* (*W.*) *xingyiense* is attributed to the *salopiense* group by the morphological characters. Karyotypically, species of the subgenus *Wilhelmia* form two distinct groups (Grinchuk *et al.*, 1972, 1974, 1975; Knoz *et al.*, 1975). Specific features of the first group, *equinum*, are the absence of the chromocenter, the presence of a distinct widened zone in chromosome Ⅰ and two distinct large puffs unusually located at the distal end of IIS, and low inversion polymorphism. The second group, *salopiense*, possesses the chromocenter, lacks a widened zone in chromosome Ⅰ, has the marker puffs in the median region of IIS, and shows high inversion polymorphism. It is clear that the species under study belongs to the second group in accordance with the result based on the morphological characteristics. Thus, chromocenter can be considered as one of the distinguishable features between species groups of the subgenus *Wilhelmia*.

Polymorphism of a natural population may be characterized by two parameters, number of individuals heterozygous for an inversion and average number of inversions per individual. In our samples from the *S.* (*W.*) *xingyiense* population, no homozygous inversion is detected. The wild population of *S.* (*W.*) *xingyiense* examined in this study appears to be heterozygous: there are 0.64 inversions per individual. Moreover, inversion polymorphism only occurs in chromosome arms IS, IL, IIL and IIIL. Compared with inversion polymorphisms of other reported species of the subgenus *Wilhelmia*, there is a marked difference neither in type of inversion, the distribution of chromosome arm nor in frequencies of individual inversions. Moreover, the present study indicates that the geographic populations of *S.* (*W.*) *xingyiense* may exist in different cytotypes.

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兴义维蚋多线染色体研究

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摘要：首次在国内对兴义维蚋 *Simulium*（*Wilhelmia*）*xingyiense* 的多线染色体进行研究，并提供其多线染色体标准图。选取兴义维蚋的成熟幼虫，用改良苯酚品红染色法进行唾腺多线染色体制备，并进行测量、描述及分析。结果表明：兴义维蚋多线染色体数目为 3 对（2n = 6）。Ⅰ号染色体具中央着丝粒，Ⅱ和Ⅲ号染色体均为亚中央着丝粒染色体。核仁组织者区位于Ⅰ号染色体短臂近着丝粒端。巴尔比尼氏环和双泡位于Ⅱ号染色体短臂近中央位置。3 对染色体的着丝粒区可形成明显的染色中心。兴义维蚋多线染色体具有多态性的倒位，倒位频率为 0.64。兴义维蚋多线染色体的着丝粒、核仁组织区、巴氏环、双泡等主要特征性结构的位置及形态恒定一致，可作为该种的重要鉴别特征。其多态性的倒位可为该蚋种在细胞水平上进行蚋类分类鉴别和系统发育等研究提供基础资料。

关键词：兴义维蚋；多线染色体；染色体图；核型；倒位多态性

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